MICROGRIPPERS TO HANDLE ORGANOIDS AND PANCREATIC ISLETS FOR PRECISION MEASUREMENTS OF BIOLOGICAL FUNCTION.

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ABSTRACT

The model of the cultured single cell is considered insufficient to explain the physiological regulation taking place at the organ level. The same is true for the prediction of drug action at the organ level or at the level of the intact organism. For these reasons 3D cell culture models are in increasing demand. It is thus necessary to develop the instruments to handle such cell aggregates and organoids in a controlled, precise and gentle manner. Here, a microgripper is presented which is able to work in aqueous solutions and which is compatible with electrophysiological recordings of the cells immobilized by it. It was successfully employed to position isolated pancreatic islets and a 3D cell culture model of insulin-secreting cells, the so-called MIN6-pseudoislet. As required it was possible to measure the membrane potential of cells within these aggregates without any interference from the microgripper.

Keywords: Diabetes mellitus, insulin secretion, Organ-on-chip, multiparametric measurements

INTRODUCTION

2D cell culture systems have been the workhorse for biomedical research for decades. However, it is increasingly becoming clear that this reductionist model of cellular physiology is insufficient to explain the regulation taking place at the organ level (**1**). In particular, it is insufficient to reliably predict the action of newly developed drugs in the intact organism (**2**). Consequently, 3D cell culture models are gaining in popularity and it is expected that more relevant information can be gathered from their use. However, the standard instrumentation in the cell biology laboratory is designed to deal with liquids and with cells suspensions but not with larger cell aggregates or organoids. In view of these tendencies it is imperative to develop the instruments to handle cell aggregates and organoids in a controlled, precise and gentle manner. Here we present the first version of a microgripper designed to deal with biological specimen.

The microgripper was used to position isolated pancreatic islets and a 3D cell culture model, the so-called MIN6-pseudoislet. Pancreatic islets contain multiple cell types, most notably the beta cells which synthesize and release insulin, the main glucoregulatory hormone of the human body (**3**). MIN6 cells are immortalized insulin-secreting cells, which share many similarities with the beta cells, but have a much higher tendency to proliferate (**4**).

RESEARCH CONCEPT

Microgripper The design of the microgripper is based on the microgripper toolbox developed at the IMT of the TU Braunschweig (**5**). To operate the microgipper in aqueous solutions and to be compatible with electrophysiological recordings of the cells immobilized by the gripper, the following design was chosen: The microgripper is fully made of SU-8, a UV sensitive, high contrast, epoxy based negative tone photoresist. Due to lithographic exposure with UV radiation and subsequent development and curing, the resin polymerizes. It is then fluid resistant and almost transparent. In order to prevent the electrophysiological investigations from being disturbed by the drive that opens and closes the gripper jaws, non-electrical actuator principles were selected and investigated. These are, on the one hand, a pneumatic and, on the other hand, a bowdencable based

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actuator principle. The investigations described here are made with the latter principle. The total length of the gripper is 7.2 mm. The length of the gripper jaws is about 700 µm, and they can be opened to 400 µm. The actuator's linear positioning movement is transmitted via an integrated gripper gear to the gripper jaws, so that they move evenly and parallel to each other, thus having a centering effect. This ensures a reliable holding process safety and an even load on the object to be gripped (**Fig. 1**).

Cells and tissues Pancreatic islets of NMRI mice were isolated from the surrounding pancreatic exocrine tissue by a collagenase digestion technique. Isolated islets were collected under a stereomicroscope and were cultured overnight in cell culture medium RPMI1640 with 10% fetal bovine serum (FBS) and 5 mM glucose. Insulin-secreting MIN6 cells (kindly provided by Jun-Ichi Miyazaki) were cultured in DMEM medium (25 mM glucose, 6 mM glutamine), 10% FBS and penicillin/streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

Monitoring of islet function parameters: The plasma membrane potential and currents of single cells within islets or pseudo-islets were measured using the patch clamp technique (**Fig. 2**). Pipettes were pulled from borosilicate glass (2 mm o.d., 1.4 mm i.d., Hilgenberg, Germany) by a two-stage vertical puller (HEKA-Electronics, Lambrecht, Germany) and had resistances between 3 and 6 MΩ when filled with solution. The continuous measurements of the membrane potential and membrane currents were performed using an EPC 7 patch-clamp amplifier (HEKA-Electronics).

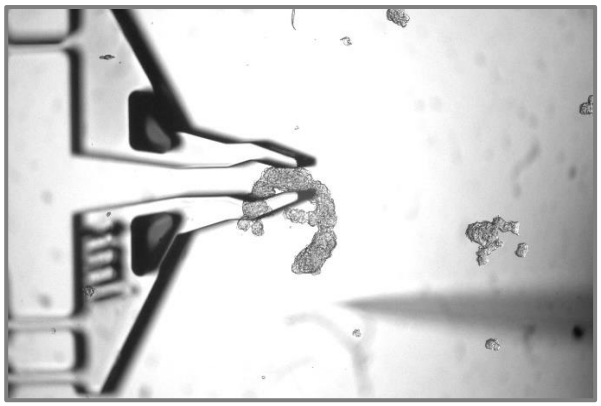

Fig. 1 Microgripper holding and moving a MIN6 pseudo-islet in the bath system of the patch clamp measuring stand

Fig. 2 Patch-Clamp measuring stand with the micro-gripper holder to the left of the organ bath (illuminated area) and the preamplifier to the right.

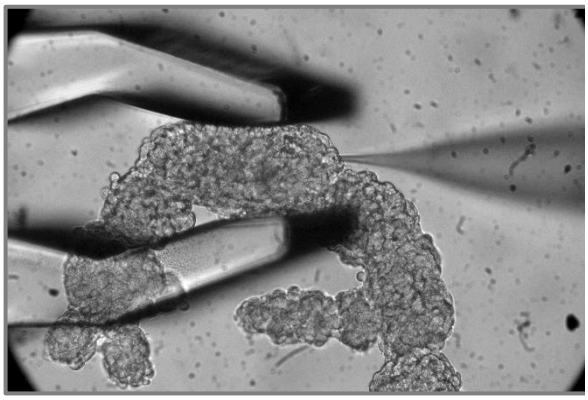

Fig. 3 Microgripper holding a MIN6 pseudo-islet while a Giga-Ohm seal is formed by the patch pipette, permitting electrophysiological measurements.

RESULTS

The jaws of the present version of the microgripper were actuated by a newly developed actuation system based on a stepper motor, a fine positioner and a bowdencable-like transmission system. With this actuation system the distance between the jaws could be adjusted extremely precise from 400 µm to a complete closed stage. This movement of the jaws generated a contact between the microgripper and the spheroid islet which was sufficiently stable to permit back and forth movements within the aqueous bath solution. The newest design of the microgripper is equipped with an integrated force

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sensor to monitor the exerted gripping-forces. The determination of the exerted force by the jaws of the gripper is subject of current investigation (a calibration facility is currently under development). But it can be stated that the exerted gripping force only slightly deformed the islet shape. When MIN6 pseudo-islets were used it was difficult to establish a sufficiently stable contact, probably because they are more easily deformable.

Placing a patch pipette (tip diameter ca. 1 µm) on one of the outer pseudo-islet cells (**Fig. 3**) and applying gentle suction (8 cm water) led to the build-up of a Giga-Ohm seal, as could be seen from the diminishing currents elicited by the -10 mV test pulse. This close connection between the cell membrane and the glass surface of the pipette is indispensable to measure the very small currents flowing through the ion channels of the plasma membrane. It is easily disturbed by even small movements of the cell surface relative to the tip of the pipette. In the present experiments a seal of more than 1 Giga Ohm persisted reproducibly for more than 15 min.

DISCUSSION

The present design of a mechanically actuated microgripper for 3D cell culture models fulfills several of the pre-defined requirements. It can generate a sufficiently firm contact to permit the positioning of the multicellular aggregates within cell culture media or other bath solutions. It does not interfere with the sensitive measurements of membrane currents and potentials. It is sufficiently stable to permit extended periods of measurement. The more pronounced difficulties in handling the pseudo-islets suggest that changes in the jaw design may be necessary to deal with mechanically less stable cell aggregates.

CONCLUSIONS

The present microgripper demonstrates the feasibility of using this kind of instrumentation to handle multicellular aggregates. In particular, a good long-term stability is achieved. This now enables further development such automated pick and place using integrated object recognition.

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